# Optimization of the Synthesis of ß-Methyltryptophan Methyl Ester and an N-Acetyl Analog of Lavendamycin Methyl Ester

An Honors Thesis

by

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**ABSTRACT**

The purpose of this thesis project was twofold. The first objective was to optimize the synthesis of ß-methyltryptophan methyl ester to improve the efficiency and yield of the overall procedure. ß-Methyltryptophan methyl ester is important in the synthesis of many antitumor anticancer drug candidates, including ß-carbolines and lavendamycin. The second objective was to synthesize one of these drug candidates, a new analog of lavendamycin called N-propionyllavendamycin methyl ester. This compound will subsequently be tested against tumor cells in vitro and in vivo in hopes that it will be an effective anticancer agent.

**ACKNOWLEDGEMENTS**

I would like to first thank Dr. Mohammad Behforouz for his great instruction, guidance, and encouragement in not just chemistry but also in life. He has been a valuable part of my education and my college experience. Also, Brian Mathes has been my lab partner for three years in both classes and in research. Although I often tease him that he couldn't have made it without me, its probably the other way around. He has been an indispensible friend and colleague in research, study, and social life.

I would also like to acknowledge the past group members who have helped me through their laboratory notebooks and advice: Wen Cai, Hammideh Zarenmayeh, Jalal Haddad, Tiffanie Lamar, Mark Stocksdale, and Sarah West. Wen Cai has been especially helpful by very patiently providing her assistance and experience.

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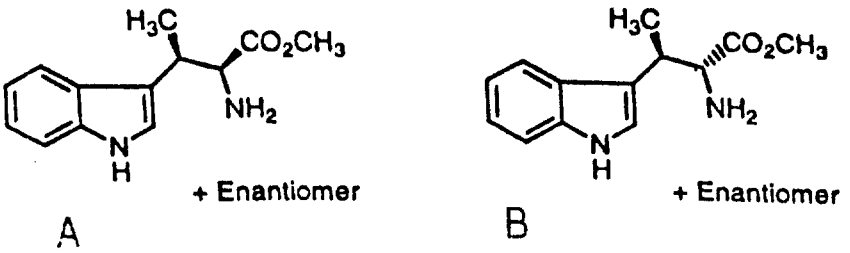
# I. ß-METHYLTRYPTOPHAN METHYL ESTER

## 1. BACKGROUND

The complete, efficient, stereoselective synthesis of the amino acid ß-methyltryptophan methyl ester is important in the synthesis of the methyl ester of the natural anti-tumor antibiotic lavendamycin. Analogs of lavendamycin, and especially the N-acetyl analogs of its methyl ester, have shown to be potent anti-tumor agents possessing high selective toxicity against cancer cells.[1]

The stereochemistry of ß-methyltryptophan methyl ester is shown below:




Isomer A includes both the 2S,3R and 2R,3S syn enantiomers.
Isomer B includes both the 2R,3R and 2S,3S anti enantiomers.

There are two methods currently in the literature for the preparation of ß-methyltryptophan methyl ester. The acetomidomalonate method, developed by Snyder in 1957, is rather long and tedious, affording only a 27% yield of mixed isomers.[3] The nitroacetate method was first developed by Lyttle in 1947,[4] then Erofeev in 1978,[5] and Rao in 1984,[6] but none of the three were stereoselective and yielded diastereomeric mixtures of A and B 47%. Finally in 1988 the nitroacetate method was modified by Behforouz through the isolation of an isomer and a change in catalyst to improve the yield to 71% from indole of pure isomer A.[7]

The emphasis on the production of pure isomer A is due to the dependence of the Pictet-Spengler condensation on the stereochemistry of ß-methyltryptophan methyl ester.[8] The proposed mechanism of the condensation requires that the most acidic hydrogen of the pi-excessive indole ring system attacks the aldamine complex to form an intermediate five-membered ring. It is important that both the methyl and the methyl ester groups are pinned back so as not to stericly hinder this attack. Whether it is the 2S,3R or 2R,3S enantiomer is not important, just that it is in the syn conformation.

Therefore, a simple stereoselective synthesis with high yields of isomer A will greatly provide for the rapid and efficient synthesis of lavendamycin methyl ester analogs against cancer. The optimization of that synthesis is reported here.

## 2. RESULTS AND DISCUSSION

### SYNTHESIS OF (2RS,3SR)-2-AMINO-3-(3-INDOLYL) BUTANOIC ACID METHYL ESTER (ß-METHYLTRYPTOPHAN METHYL ESTER)

The major objective of this part of the project was to optimize the synthesis of ß-methyltryptophan methyl ester to improve the efficiency and the yield of the overall procedure. There were two particular steps that needed attention, the crystalization of the nitro compound (5) and the formation of an unwanted nickel salt in the final product (6). These will be elaborated on in the following discussion of the procedure.

3-(Isopropylaminoethylidene)indole (3)

The process begins with the reaction of indole (1) with ethylideneisopropylamine (2). The reaction is proposed to proceed through electrophillic attack on the double bond by the most reactive proton on the pi excessive indole ring system. This is an exothermic reaction requiring slow addition and an ice bath to keep the temperature below 15°C. Purification affords light brown crystals of (3) 70%.

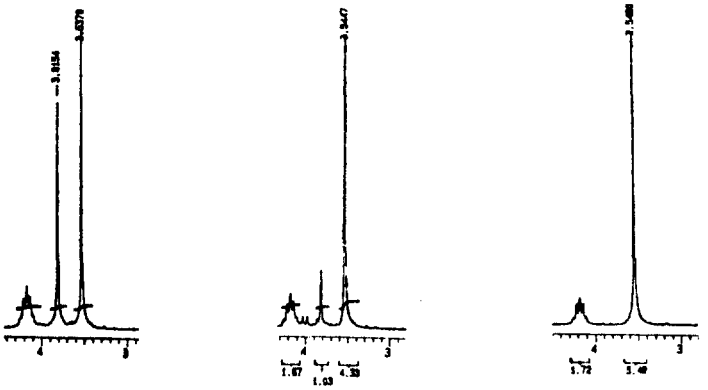
Methyl(2RS,3SR)-2-nitro-3-(3-indolyl)butanoate (5)

The next step is the exchange of the isopropylaminoethylidene group with methyl nitroadetate. This is proposed to proceed by the formation of the methyl nitroacetate carbocation facilitated by the triethylamine. This carbocation provides a proton to the nitrogen of the isopropylamine , making it a good leaving group, and allowing nucleophilic substitution to proceed.

The crystallization of the final nitro compound proved difficult because this is the step where stereochemistry is introduced into the reaction. It turns out that when the ratio of the isomers A & B (as labeled in the BACKGROUND section) is near equal in the reaction mixture, a thick, dark residue forms rather than the desired off-white crystals. The first attempts to solve this problem included changing reaction conditions such as temperature, temperature ramp, and time, but no consistent change was seen. Next a lower percentage of hydrochloric acid was used

to acidify the reaction mixture (10% to 5%). This was helpful, but did not solve the problem.

Finally, it was discovered that by redissolving the residue in a chloroform hexane and then reevaporating it three times, the ratio of isomers changed with each successive evaporation to yield more of isomer A. This can be followed nicely by 1H NMR as shown below. It appears that isomer A is both less soluble than isomer B and slightly more stable, because not only does more isomer A crystalize out with each evaporation, but it does not appear to revert back to B when dissolved into solution again. A final recrystallization gives only isomer A (87%).



Methyl(2RS,3SR)-2-amino-3-(3-indolyl)butanoate (6)

The final reaction in the synthesis of ß-methyltryptophan methyl ester is the reduction of isomer A of the nitro compound. This is a simple hydrogenation reaction using Raney Nickel catalyst in the presence of trifluoroacetic acid which maintains the stereochemistry of the reactant in the product and solely reduces the nitro group to an amine.

The formation of a green nickel salt in the purification of the final product made separation and filtration difficult and yields low. It is proposed that the trifluoroacetic acid is strong enough to ionize the nickel metal from the catalyst to nickel ion, which was then forming a salt or a nickel oxide with the sodium carbonate previously used to basify the reaction mixture. This nickel salt was a green emulsion which would visibly trap some of the product during filtration, yet was virtually undissolvable. It also left green nickel complexes in

the organic layer which required large amounts of ammonium chloride to remove.

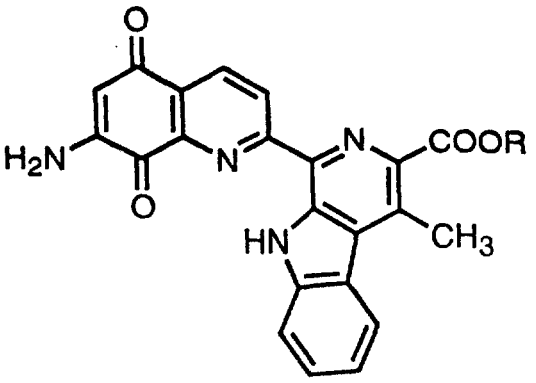
The first change made was to reduce the amount of excess trifluoroacetic acid from 6:1 to 2:1 to prevent nickel ion formation. in addition, the sodium carbonate was replaced by ammonium hydroxide for basifying the reaction mixture. This forced any nickel ion present to form an inorganic nickel hydroxide complex which easily dissolved in the aqueous layer, making a clear blue layer which was well distinguished from the yellow organic layer. Thus the purification was made easier and yields increased to 96%.

With these two more significant changes plus other smaller tweaks of the procedure, a greater overall yield was achieved. More importantly, the synthesis was made much cleaner, straightforward, and thus more enjoyable once the more difficult steps were simplified. This affords the rapid and efficient synthesis of ß-methyltryptophan methyl ester, which ultimately improves the efficiency of synthesizing ß-carbolines and antitumor antibiotic analogs such as lavendamycin methyl ester.

## II. N-PROPIONYLLAVENDAMYCIN METHYL ESTER

### 1. BACKGROUND

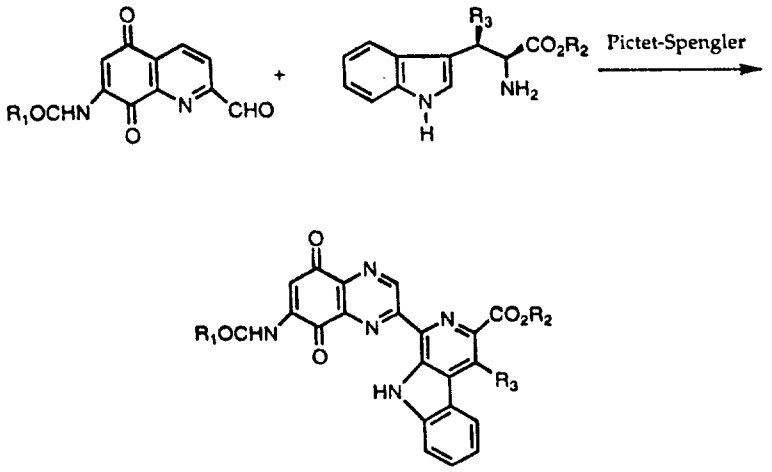
Lavendamycin was first isolated by Doyle in 1981 from the fermentation broths of the bacteria *Streptomyces lavendulae* at Bristol Laboratories.[9] Lavendamycin is a dark red solid with very limited solubility and a structure (shown below) similar to that of Streptonigrin.




Like Streptonigrin, Lavendamycin is an antitumor antibiotic, but both have previously been unsuccesful as antitumor agents because of their high toxicity.[10] However, more recently, the N-acetyl analogs of lavendamycin methyl ester have shown very promising in vitro and in vivo selective cytotoxicity at unprecedented levels compared to the current antitumor agents available.[1] Changing the structure or the electronics of the N-acetyl substitutions on the Lavendamycin methyl ester ring system may positively affect this antitumor activity by increasing effectiveness while decreasing toxicity.

The first total synthesis of Lavendamycin methyl ester was by Kende in 1984.[11] The synthesis began with the condensation of a quinaldic acid with ß-methyltryptophan methyl ester to give an amide which was then converted to a ß-carboline ester by a Bischler-Napieralski condensation.[13]

A more current synthesis developed by Behforouz uses the Pictet-Spengler condensation to condense ß-methyltryptophan methyl ester with various quinolinedione aldehydes to yield the final product in one step.[12] This procedure eases the synthesis of Lavendamycin methyl ester analogs because the aldehyde can be manipulated and substituted relatively easily before condensation to the final product. The general condensation reaction is shown below:




The synthesis of ß-methyltryptophan methyl ester has been reported in Section 1. Here the synthesis of the N-propionyl substituted quinolinedione aldehyde and its condensation with ß-methyltryptophan methyl ester is reported to yield a new analog of Lavendamycin methyl ester. This furthers our group's work toward synthesizing various analogs which may show increased activity as antitumor agents against cancer cells.

## 2. RESULTS AND DISCUSSION

### SYNTHESIS OF 7-PROPIONYLLAVENDAMYCIN METHYL ESTER

The objective of this part of the project was to synthesize a new N-acetyl analog of lavendamycin methyl ester by the Pictet-Spengler condensation of ß-methyltryptophan methyl ester and 7-propionamido-2-formylquinoline-5,8-dione. The aldehyde was synthesized first starting from 8-hydroxy-2-quinoline and then condensed with ß-methyltryptophan methyl ester from the first part of this project.

5,7-diamino-8-hydroxy-2-methylquinoline hydrochloride (1)

This compound was made from 8-hydroxy-2-methylquinoline by nitration using a combination of concentrated nitric and sulferic acid in an ice bath, and then reduction over Paladium charcoal in the presence of hydrochloric acid. Both reactions are straight forward and high yielding to give 90% bright orange crystals.

5,7 Dipropionamido-8-hydroxy-2-methylquinoline (3)

Propionic anhydride (2) was reacted with (1) in the presence of sodium acetate and sodium sulfate. This produced N-propionyl groups at the 5 and 7 positions and often added to the 8-hydroxy position as well until recrystallization in methanol and water produced complete (3). Before recrystalization the filter cake is a pasty grey material, but recrystaliztion yield clean white crystals which are cubic in shape (89%). The reaction is controlled by an equilibrium such that once the first precipitate product is filtered off the reaction mixture can be stirred again to yield more product.

7-Propionamido-2-formylquinoline-5,8-dione (5)

The aldehyde is completed by a series of two oxidations. First 7-propionamido-2-methylquinoline-5,8-dione (4) is formed through a simple oxidation by potassium dichromate in acetic acid. Then the methyl group in the 2 position is oxidized to the aldehyde by refluxing under Argon with selenium dioxide in 1,4

dioxane at 110°C. The final product is bright yellow with a needle crystal structure (79%).

N-Propionyllavendamycin methyl ester (7)

The Pictet-Spengler condensation of the aldehyde (5) and ß-methyltryptophan methyl ester (6) was carried out under Argon in refluxing xylene at 130°C. No other reagent or catalyst is needed, and the reaction goes to completion within 48 hrs. It can easily be monitored by thin layer chromatography to determine completion, and affords the characteristic dark red solid of lavendamycin methyl ester.

The synthesis of the new N-acetyl analog of lavendamycin was a relatively simple procedure of oxidations and reductions and a final condensation. This serves to demonstrate the efficiency of the single isomer A of ß-methyltryptophan methyl ester and to add yet another N-acetyl analog to those being tested in the search for an antitumor drug candidate with high cytotoxicity.

## III. EXPERIMENTAL

### 1. GENERAL EXPERIMENTAL

REAGENTS: acetaldehyde, isopropylamine, indole, methyl nitro acetate, Raney nickel catalyst, 8-hydroxy-2-methylquinoline, propionic anhydride, paladium charcoal, and selenium dioxide were all purchased from Aldrich Chemical Company.

SOLVENTS: 1,4 dioxane, toluene, and xylene were dried and distilled before use. Chloroform, ethyl acetate, ethyl ether, dichloromethane, hexane, and 95% ethanol were reagent grade and not distilled.

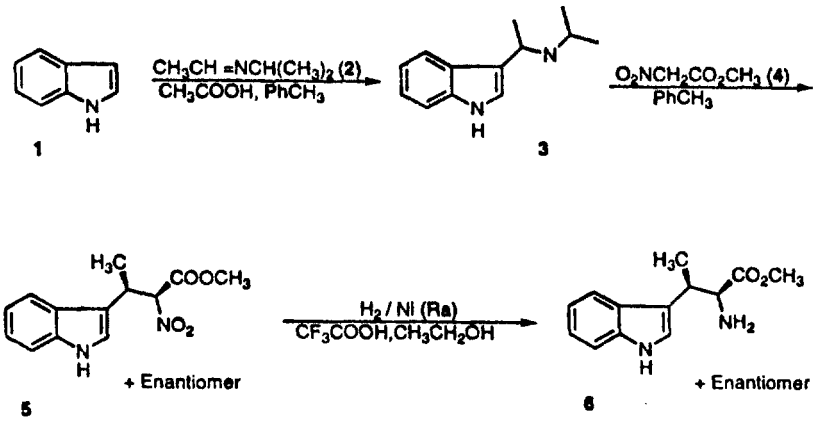
MELTING RANGES: Melting ranges were recorded with a Thomas Hoover capillary melting point apparatus.

THIN LAYER CHROMATOGRAPHY: TLC was performed using Eastman Kodak silica gel sheets with flourescent indicator.

NUCLEAR MAGNETIC RESONANCE (1H NMR): NMR spectra were recorded on a Varian Gemini 200 spectrometer in deuterated chloroform or deuterated dimethyl sulfoxide (DMSO) with tetramethyl silane (TMS) or residual DMSO as the internal standards.

## 2. PROCEDURAL EXPERIMENTAL

### 2 A. SYNTHESIS OF (2RS,3SR)-2-AMINO-3-(3-INDOLYL) BUTANOIC ACID METHYL ESTER (ß-METHYLTRYPTOPHAN METHYL ESTER)




*A. Ethylideneisopropylamine (2)* A 1-L, three-necked, round-bottomed flask, equipped with a mechanical stirrer, dropping funnel, thermometer, and condenser cooled with ice-water and protected from moisture by a calcium chloride-filled drying tube, is immersed in an ice-salt bath. In the flask is placed isopropylamine (59.0 g, 1.0 mol). Freshly distilled acetaldehyde (44.0 g, 1.0 mol) is added at 0°C over a period of 3 hrs with stirring. The colorless solution is stirred for an additional 20 min. The reaction mixture is transferred to a 500 mL Erlenmeyer flask and ground potassium hydroxide flakes are added until separation into two layers occurs. The light-brown upper organic layer is separated from the dark-brown lower aqueous layer and allowed to stand over barium oxide in the cold room overnight. The resulting brown mixture is filtered to obtain the colorless liquid (Note 1) which is stored tightly stoppered in the refrigerator: 71.89 g (84 %).

*B. 3-(Isopropylaminoethylidene)indole (3).* A 1-L, three-necked, round-bottomed flask, equipped with a mechanical stirrer, dropping funnel, thermometer, and condenser cooled with ice-water and protected from moisture by a calcium chloride-filled drying

more times to yield brown colored crystals which were recrystallized in the same chloroform: hexane mixture to yield light brown to off-white crystals of isomer A (Note 5): 22.94 g (87 %).

*D. Methyl(2RS,3SR)-2-Amino-3-(3-indolyl)butanoate (6).* In a 500 mL heavy-walled flask containing a magnetic stir bar is placed an ice cooled solution of trifluoroacetic acid (2.6 g, 22.8 mmol) in 150 mL absolute ethanol to which ground nitroester (4) (3.0 g, 11.4 mmol) is added. The mixture is stirred for 30 min to dissolve most of the solid. The magnetic stir bar is removed and 9 g of commercial Raney nickel catalyst is added after having been rinsed with absolute ethanol (3 x 50 mL). Hydrogenation is carried out at 40 psi (2.75 atm) at room temperature until hydrogen absorption ceased (1 hour). The reaction mixture is filtered carefully through a layer of celite (Note 6) and the filter cake washed with absolute ethanol (3 x 50 mL). The filtrate is then evaporated to yield a yellow-green liquid. To this material 100 mL ether, 50 mL water, and 15 mL 14 % ammonium hydroxide were added. The yellow upper ether layer is separated from the blue lower aqueous layer and saved. The aqueous layer is extracted with ether (4 x 50 mL) and the ether extracts are combined with the previous ether layer and washed with water (1 x 100 mL) and 10 % ammonium chloride (2 x 100 mL) (Note 7). After drying over magnesium sulfate, the ether is evaporated to give off-white crystals (Note 8): (2.53 g. 96 %).

NOTES

1. Distillation is not necessary since the liquid is generally very pure. If distillation is desired, it should be carried out under very low heat and vacuum of about 125 mm Hg.

2. Purification is difficult and yield low when previously opened or less than 99 % pure indole is used.

3. Methyl nitroacetate is commercially available but expensive. It can easily be prepared by the method found in *J.Heterocyclic.Chem.*, 1988, **25**, 1627.

4. The liberation of isopropylamine can be checked with pH paper during the process.

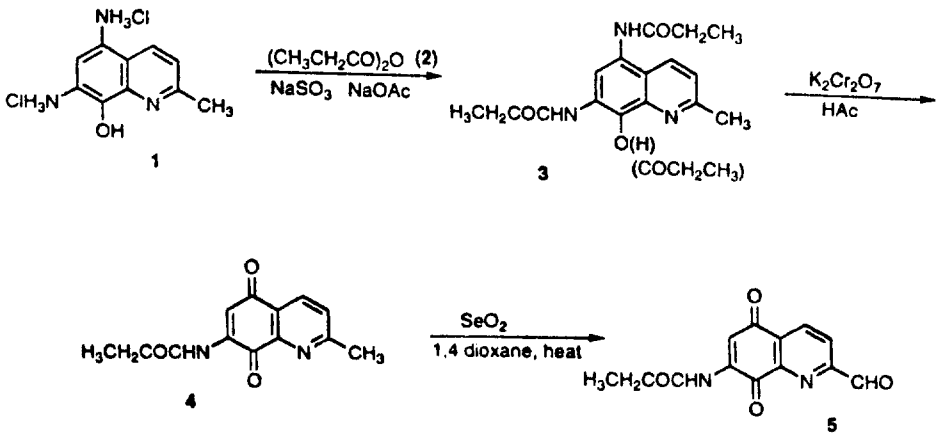
5. The desired isomer A appears to be more stable than

isomer B, such that repeated evaporations and a final recrystallization promote the conversion between isomers favoring isomer A. As the ratio of isomers changes with successive treatments, the residue changes from a thick, dark, oily substance to the desired brown crystals.

6. Caution! Care must be taken in filtering to prevent the Raney nickel catalyst filter cake from becoming dry and igniting.

7. If green color persists, continue washing with ammonium chloride to remove any remaining nickel salt.

## 2B. SYNTHESIS OF 7-PROPIONAMIDO-2-FORMYLQUINOLINE-5,8-DIONE




A. *5,7 Diamino-8-hydroxy-2-methylquinoline hydrochloride (1).* In a 500-mL heavy-walled flask is placed 100 mL water and 13 mL concentrated hydrochloric acid to which 5,7-dinitro-8-hydroxy-2-methylquinoline (6.23 g, .025 mol) and 1 g Paladium charcoal catalyst are added. Hydrogenation is carried out at 30 psi overnight (about 15 hrs.). The reaction mixture is then filtered to remove the catalyst, and the filter cake washed with water (3 x 100 mL). The orange-red filtrate is evaporated and the solid dried under vacuum overnight to yield bright orange crystals: 5.90 g (90%).

B. *5,7 Dipropionamido-8-hydroxy-2-methylquinoline (3).* In a 125-mL Erlenmeyer flask equipped with magnetic stir bar is placed 5,7-diamino-8-hydroxy-2-methylquinoline hydrochloride (1.0 g, .0038 mol) dissolved in a minimum amount of water. While stirring, the following are added in rapid succession: sodium sulfite (2.40 g, .019 mol), sodium acetate (3.10 g, .023 mol), and propionic anhydride (12.37 g, .095 mol). The solution is stirred for an additional 3 hrs. and the white precipitate filtered off, washed with water. The resulting pasty filter cake is recrystallized in methanol and water and dried under vacuum to yield white crystals (Note 1): 1.02 g (89%).

C. *7-Propionamido-2-methylquinoline-5,8-dione (4).* In a 250-mL Erlenmeyer flask is suspended 5,7-dipropionamido-8-hydroxy-2-methylquinoline (.66 g, .0017 mol) in 32 mL glacial acetic acid.

To this a solution of potassium dichromate (2.4 g, .008 mol) in 32 mL water is added and the mixture stirred for an additional 2 hrs (Note 2). 20 mL dichloromethane is then added and the mixture allowed to stir overnight. The resulting solution is washed with dichloromethane (12 x 25 mL), the organic layers combined and washed with 3% sodium bicarbonate (2 x 50 mL). The yellow solution is then dried over magnesium sulfate and evaporated to yield bright yellow crystals: .290 g (69%).

D. *7-Propionamido-2-formylquinoline-5,8-dione (5).* In a 50-mL round bottom flask equipped with magnetic stir bar, water cooled condenser, Argon filled balloon, and immersed in an oil bath is placed 7-propionamido-2-methylquinoline-5,8-dione (.200 g, .0008 mol) and selenium dioxide (.12 g, .001 mol) in 10 mL freshly distilled 1,4 dioxane and 3 drops of water (Note 3). The reaction mixture is heated to 110°C over 1 hour and allowed to reflux for an additional 25 hrs., monitored for completion by thin layer chromatography. The mixture is filtered hot to remove the selenium metal which is rinsed in hot 1,4 dioxane (5 x 20 mL). The filtrates are stored overnight at 4°C. After adding 100 mL dichloromethane, the solution is washed with 3% sodium bicarbonate (2 x 100 mL), dried over magnesium sulfate, and evaporated to yield yellow crystals: .169 g (79%).

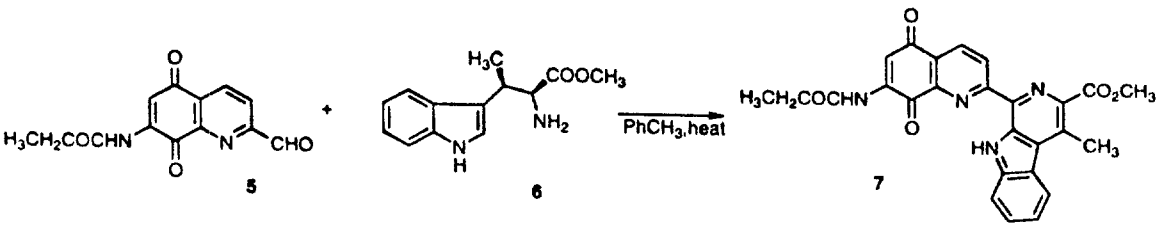
NOTES

1. After filtering off the original precipitate, further stirring of the filtrate often leads additional product.

2. The reaction mixture begins as an orange suspension but changes to a uniformly dark and clear solution.

3. The 1,4 dioxane must be freshly distilled. It is the purity of the solvent, not necessarily the dryness, which is important in this reaction. With an impure solvent the reaction does not go to completion.

## 2 C. SYNTHESIS OF 7-N-PROPIONYLLAVENDAMYCIN METHYL ESTER




In a 50-mL round bottom flask equipped with magnetic stir bar, water cooled condenser, Argon filled balloon, and immersed in an oil bath, is placed ß-methyltryptophan methyl ester (.05 g, .0002 mol) and 7-propioamido-2-formylquinoline-5,8-dione (.055 g, .00025 mol) in 20 mL freshly distilled xylene. The solution is heated to 130°C and allowed to reflux for 48 hrs. while reaction progress is monitored by thin layer chromatography. The resulting reaction mixture is filtered hot to remove the precipitate, and the filtrate evaporated to yield dark red crystals.

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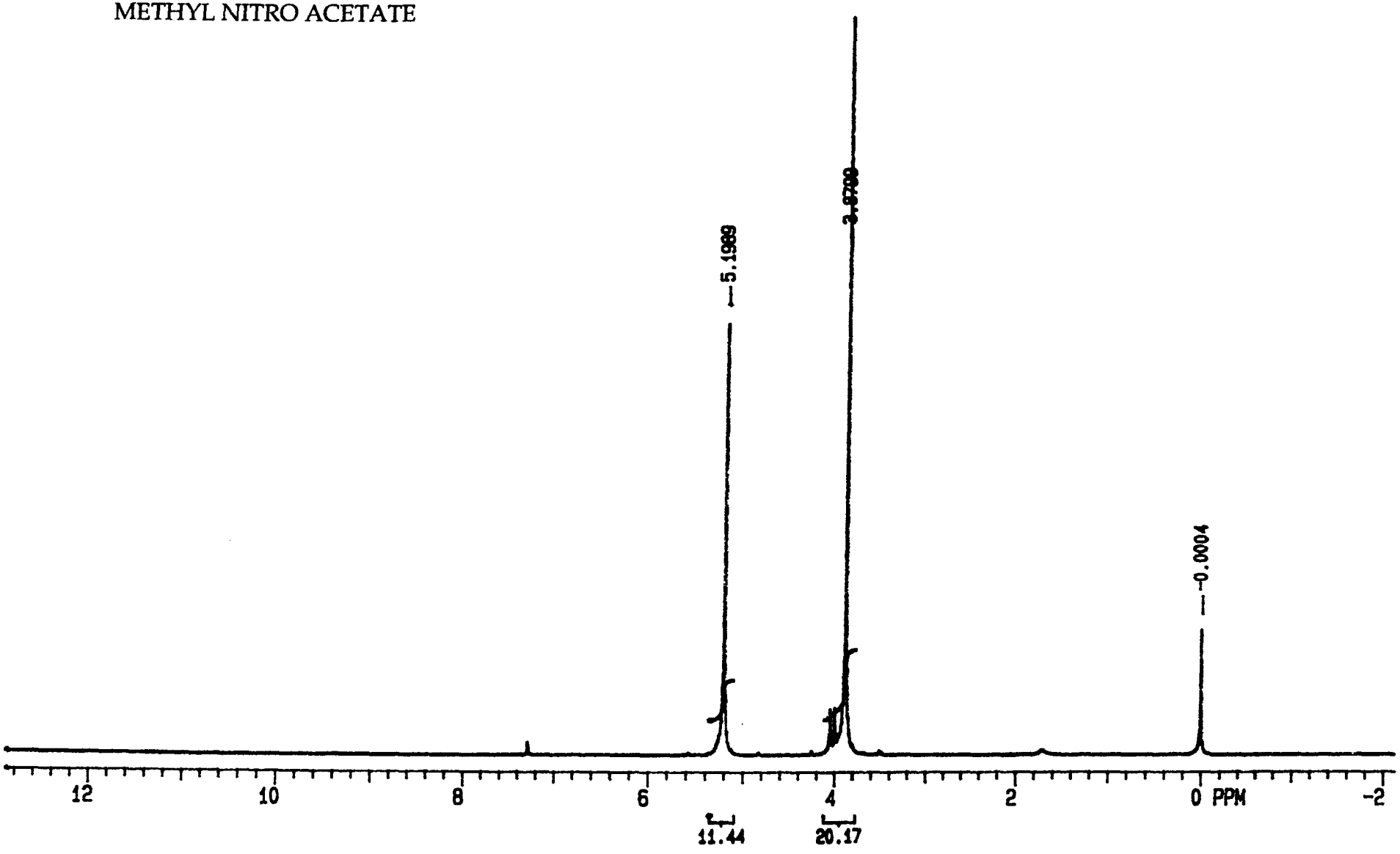
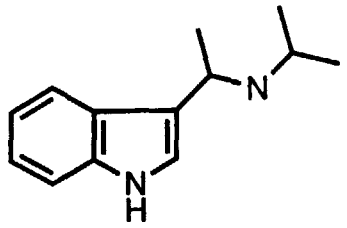
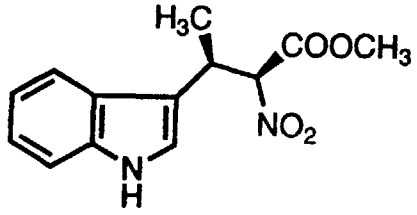
$O_2NCH_2CO_2CH_3$

METHYL NITRO ACETATE

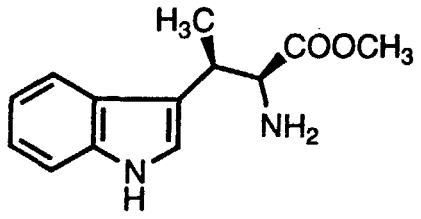




3-(ISOPROPYLAMINOETHYLIDENE) INDOLE




METHYL(2RS,3SR)-2-NITRO-3-(3-INDOLYL)BUTANOATE

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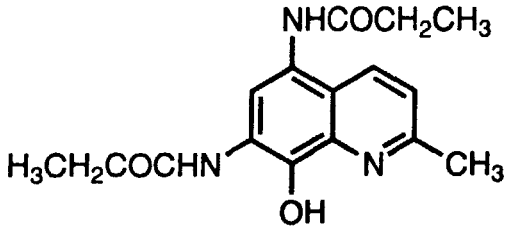
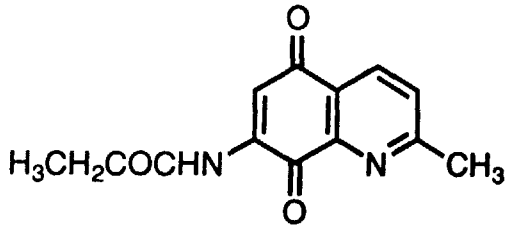
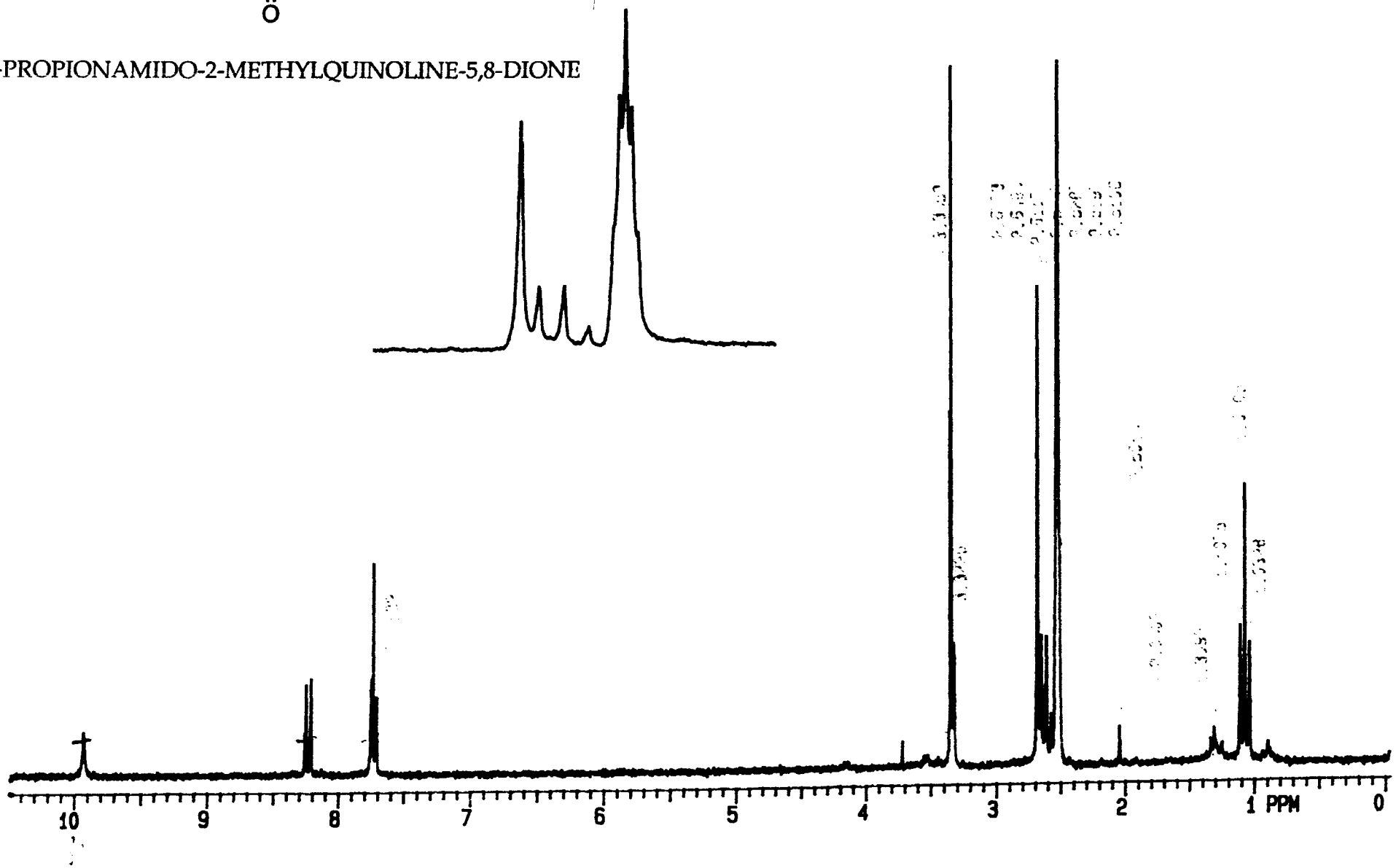
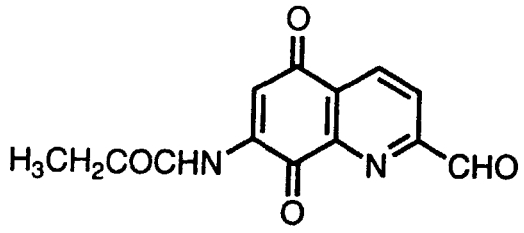



5,7 DIPROPIONAMIDO-8-HYDROXY-2-METHYLQUINOLINE




7-PROPIONAMIDO-2-METHYLQUINOLINE-5,8-DIONE






7-PROPIONAMIDO-2-FORMYLQUINOLINE-5,8-DIONE